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16. ABSTRACT

The relative toxicity of marine sediment can be accurately determined through acute, static bioassays with the phoxocepalid amphipod Repoxynius abronius. Mortality and sublethal effects on emergence from sediment and reburial behavior are determined after ten day exposure in 1-L beakers containing 175 ml of test sediment, 775 ml of seawater(25 ppt, 15°C), and 20 amphipods. Response of amphipods to test sediment is compared with response in control sediment collected from the species' natural habitat. Mean survival under control conditions is 95%. With five replicates, the bioassay is 75%certain of detecting statistical significance when mean survival is reduced by 15%. The method can be applied to a great variety of sediment types because of the tolerance of R. abronius to a broad range of sediment grain sizes and levels of organic enrichment. The bioassay can be applied to determine(1)the toxicity of sediment subject to regulatory decision (e.g. dredging or disposal),(2)the spatial distribution of sediment toxicity along pollution gradients or near point sources, and (3)the LC_{50} of contaminants added to unpolluted sediment. The species' sensitivity to low salinity limits the method to sediment from the coastal zone and lower portion of estuaries. A detailed description of the bioassay procedures is appended to this report.

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ABSTRACT: The relative toxicity of marine sediment can be accurately determined through acute, static bioassays with the phoxocephalid amphipod, Rhepoxynius abronius. Mortality, and sublethal effects on emergence from sediment and reburial behavior are determined after ten day exposure in one-litre beakers containing 175 ml of test sediment, 775 ml of seawater (25 ppt, 15°C), and 20 amphipods. Response of amphipods to test sediment is compared to that in control sediment collected from the species' natural habitat. Mean survival under control conditions is 95 percent. With five replicates the bioassay is 75 percent certain of detecting statistical significance when mean survival is reduced by 15 percent. The method can be applied to a great variety of sediment types because of the tolerance of R. abronius to a broad range of sediment grain sizes and levels of organic enrichment. The bioassay can be applied to determine (1) the toxicity of sediment subject to regulatory decision, e.g., dredging or disposal, (2) the spatial distribution of sediment toxicity along pollution gradients or near point sources, and (3) the LC50 of contaminants added to unpolluted sediment. The species' sensitivity to low salinity limits the method to sediment from the coastal zone and lower portion of estuaries. A detailed description for the bioassay procedures is appended to this report.

KEY WORDS: amphipods, bioassay, sediment, toxicity test, aquatic toxicology, invertebrates, Rhepoxynius abronius, marine pollution

INTRODUCTION

Many pollutants released into the marine environment eventually accumulate in benthic ecosystems. Research on the toxicity of contaminated sediment to benthic organisms is therefore needed in marine pollution ecology [1]. Chapman and Long [2] recommended sediment bioassays along with other methodologies to provide information on the ecological impact of contaminated sediment. One of the first marine sediment bioassays was developed in our laboratory [3] and was the basis for the solid phase bioassay procedure in the EPA/COE [4] manual for dredged material evaluation. The objective of the present report is to describe research through which that bioassay has evolved into a simpler, more reliable laboratory test of sediment toxicity. A detailed description of the new procedure is included in this report (Appendix A).

The revised method is an acute static bioassay of sediment toxicity to the infaunal, phoxocephalid amphipod, Rhepoxynius abronius. R. abronius is a burrowing species that is appropriately sensitive to the quality of interstitial water and sediment particulates. Amphipods are exposed for ten days to test and control sediment in one-litre beakers. Response criteria include mortality, emergence from sediment during exposure, and inability to rebury in clean sediment at the end of the bioassay. Test sediment can be collected from potentially contaminated field sites, or prepared by various manipulations of uncontaminated sediment in the laboratory.

METHOD DEVELOPMENT

Bioassay Species:

The species used in this bioassay is Rhepoxynius abronius Barnard (Amphipoda, Phoxocephalidae). This amphipod has previously been identified as Paraphoxus epistomus [3,5]. but the genus Paraphoxus has been revised and the correct identification is now Rhepoxynius abronius [6, 7; J. L. Barnard, personal communication].

Amphipods of the family Phoxocephalidae are the second most abundant group of crustaceans, apart from the class Ostracoda, in the soft bottom benthos of the eastern Pacific Ocean [8]. The genus Rhepoxynius consists of fifteen North American species found from Puget Sound, Washington to Baja California and from New Hampshire to South Carolina [7]. R. abronius is known from Puget Sound to southern California [6,8,9]. This species inhabits clean sands on the continental shelf and in higher salinity portions of estuaries. It occurs in the lower intertidal and subtidal zones of Yaquina Bay, Oregon although it is typically a subtidal species found to depths of 274 m [7].

The annual range in density of R. abronius at our collection site on the south shore of Yaquina Bay, Oregon (44° 37.6' N, 124° 3.0' W) is 800-2200 amphipods per m^2 (Swartz et al., unpublished data). Our observations show that R. abronius is essentially an annual species but reproductive and growth seasons are quite long and there is a temporal overlap of about nine months between succeeding generations. Peak recruitment of post-brood juveniles occurs from February through July. They reach maturity from October through January. More than 50 percent of mature females are ovigerous from December through July. After July the year class gradually

dies off and is replaced by the next generation. This seasonal cycle of growth and reproduction is similar to that described by Hannan et al. [10] and Oliver et al. [5] for Rhepoxynius from Monterey Bay, California.

Mature amphipods (3.0-5.0 mm total length) are used in the sediment bioassay. The annual range in density of this size class in Yaquina Bay is 150-750 amphipods per m^2, therefore adequate supplies of bioassay specimens are available throughout the year.

Phoxocephalid amphipods appear to have an important functional role in benthic ecosystems. Amphipods are the principal benthic prey of many fishes [11]. Phoxocephalids were the dominant burrowing invertebrate prey of seven of eight fish species studied by Manzanilla and Cross [12]. In laboratory experiments we found that the first crab instar of Dungeness crab (Cancer magister) was an efficient predator of R. abronius that had been allowed to bury before the crabs were introduced. Oliver et al. [13] identified phoxocephalids as important infaunal predators of small benthic invertebrates. Gut contents of abundant macroinvertebrates that co-occurred with the phoxocephalids contained few or no invertebrate prey. Phoxocephalids thus occupy an unusual and functionally important trophic position as the prey of the epibenthos and predator of the meiobenthos. The sensitivity of phoxocephalid amphipods to sediment contaminants is indicated by both laboratory experiments and field observations. Mearns and Word [14] included three phoxocephalids among seven invertebrate genera that dominate the soft bottom macrobenthos in control or background areas of the southern California Bight. Their abundance decreases in relation to proximity to sewage outfalls. Similarly, the phoxocephalid Rhepoxynius hudsoni (= Trichophoxus epistomus) is abundant in unpolluted areas of the New York Bight, but is not found near a sewage sludge dump site [15]. A group of seven species of phoxocephalids including R. abronius was

ubiquitous in the deeper parts of Commencement Bay, Washington but absent from adjacent highly industrialized waterways. In agreement with the field distribution of phoxocephalids and other amphipods, sediment bioassays using the Appendix A procedure showed that sediment from deeper Commencement Bay was not acutely toxic, but sediment from the waterways was often highly toxic to R. abronius [9]. R. abronius is more sensitive to contaminated sediment than a variety of benthic species including some bivalves, oligochaetes, polychaetes, amphipods and cumaceans [3; P. M. Chapman, personal communication].

In summary, R. abronius and other phoxocephalids have a wide geographic distribution, are ecologically important and are appropriately sensitive to sediment contamination. This, and possibly other phoxocephalids, are ideal test species for sediment toxicity bioassays.

Bioassay Chamber and Facilities

The bioassay chamber is a standard one-litre glass beaker with a 10-cm internal diameter. About 175 ml of test sediment are placed in the bottom to create a 2-cm deep layer. Beakers are filled to 950 ml with 25 ppt seawater, covered with a watchglass, and placed in a 15°C water bath. Constant illumination is provided by overhead lights. Water in the beakers is aerated without disturbing the sediment surface. Twenty amphipods are placed in each beaker. The bioassay is terminated after ten days of exposure. The scale of this system presents minimal logistical difficulty and allows adequate replication for most statistical designs. Sixty beakers can conveniently be included in one experiment, although some bioassays have involved as many as 270 beakers (P. M. Chapman, personal communication).

The static seawater system represents a worst case in the sense that toxic materials leached from the sediment will not be washed away. In fact there is relatively little transport of water, salt, or toxic materials across the sediment-water boundary in these beakers. Small infaunal amphipods like R. abronius do not require large or elaborate facilities to simulate their natural habitat. That simulation is much more dependent on the quality of the sediment rather than the seawater system.

The density of amphipods in the beaker is slightly greater than that at the collection site, but effects due to intraspecific interactions have never been evident. In nature phoxocephalid amphipods may migrate into the water column at night for purposes of reproduction and dispersion [10]. This does not normally occur in the laboratory under constant illumination. R. abronius can burrow as deep as 6 cm but normally inhabits the upper 2 cm (W. G. Ambrose, personal communication). The 2-cm depth of test sediment is therefore adequate for this species.

Response Criteria

Survival - The primary response criterion is survival after ten days exposure to test or control sediment. Control sediment is collected at the same site as the test specimens and therefore assumed to be nontoxic. A permanent and apparently very healthy population of R. abronius exists in Yaquina Bay, Oregon. Survival in control sediment from Yaquina Bay averaged 19.0 of 20 amphipods in 22 experiments, each with five control replicates. Survival in control sediment was less than 17 individuals in only 2 of 110 instances. The lowest mean control survival for an individual experiment was 17.8 amphipods.

The precision of the bioassay is dependent on the number of replicates and the number of individuals (Table 1). With five replicates per treatment and 20 amphipods per replicate the bioassay is 75 percent certain of detecting statistical significance ($p < 0.05$) if the difference in mean survival between control and test sediment is 2.8. For normal control survival (19.0), this corresponds to a test sediment mean survival of 16.2, about a 15 percent reduction. Since survival in test sediment is often much less than 16 [3, 9], this is a reasonable level of precision for most applications.

When a bioassay is terminated the test sediment is sieved through a 0.5-mm screen and the number of live and dead amphipods is counted. Missing animals are assumed to have died and decomposed (Appendix A). This assumption was tested by monitoring decomposition of dead animals. Healthy amphipods were sacrificed by a 30 min exposure to 35°C seawater. Ten dead individuals were placed in bioassay beakers containing a 2-cm layer of control sediment. They were then buried beneath 1-3 mm of sediment. Seawater was added to the beakers and they were placed in a 15°C water bath. After 0, 1, 3, 4 and 7 days, the contents of one of the beakers were sieved and the number of intact bodies, heads and tails was recorded. Ten whole bodies were recorded at the initiation of the experiment and after one day. Five whole bodies, five heads and five tails were found after three and four days. After seven days no whole bodies were recovered. Nine heads and seven tails in advanced stages of decomposition were found. Thus the assumption of loss through death and decomposition was confirmed.

Reburial - At the end of the exposure survivors are placed in bowls containing control sediment and uncontaminated seawater and the number that are unable to rebury within one hour is recorded. Amphipods are counted as survivors even if they are able only to twitch a pleopod. The reburial

Table 1. Precision of the benthic bioassay in relation to sample size and replication. δ is the difference between two survival means for which the bioassay is 75 percent certain of detecting statistical significance ($p < 0.05$) [16]. δ/c expresses the precision estimate as a percent of the normal control survival ($c = 19.0$ for $n = 20$, $c = 9.5$ for $n = 10$).

Number of Amphipods per Replicate					
10			20		
Number of Replicates	δ	δ/c	Number of Replicates	δ	δ/c
2	6.80	71.6			
4	2.66	28.0	2	8.55	45.0
6	1.94	20.4	3	4.44	23.4
8	1.60	16.8	4	3.35	17.6
10	1.38	14.5	5	2.80	14.7
12	1.25	13.2	6	2.45	12.9
14	1.14	12.0	7	2.20	11.6
16	1.05	11.0	8	2.02	10.6
18	0.98	10.3	9	1.89	10.0
20	0.93	9.8	10	1.76	9.3

test is included in the bioassay procedure to discriminate such individuals from healthy, active amphipods. Inability of an infaunal animal to rebury increases its vulnerability to predation and has obvious biological significance for individuals and, possibly, populations.

Of 8975 survivors from all treatments in bioassays that we have conducted, 8747 (97.5 percent) were able to rebury. Although failure to rebury is uncommon, it is not evenly distributed among all treatments. The proportion of survivors that do not rebury increases significantly ($p < 0.01$) as the number of survivors decreases (Table 2). Failure to rebury is low (<1 percent) among amphipods from treatments where survival was high ($\geq$16 of 20 individuals). About 20 percent of amphipods failed to rebury when survival was low ($\leq$5 of 20 individuals).

Emergence from Sediment - Since most amphipods remain buried during sediment bioassays, there are limited opportunities to monitor temporal changes in survival or sublethal effects. An exception is the temporal pattern of emergence of amphipods from toxic sediment. Data are collected daily by counting the number of amphipods either partially or totally emerged from the sediment. Few animals emerge from clean sediment under bioassay conditions. A mean of only 0.3 of 20 amphipods emerge in ten days in beakers containing control sediment. In contrast, many amphipods may emerge from sediment containing toxic materials. For example, in experiments in which cadmium was added to Yaquina Bay control sediment, up to 60 percent of the amphipods emerged in one treatment and the emergence rate was proportional to the concentration of cadmium in sediment (Fig. 1). The emergence rate of R. abronius was essentially constant over ten days at 8 mg/kg cadmium (dry weight), but decreased rapidly after six days at 16 mg/kg. Amphipods will emerge within seconds from sediment conditions they seem able to sense, e.g., interstitial water salinity < 5 ppt. The much

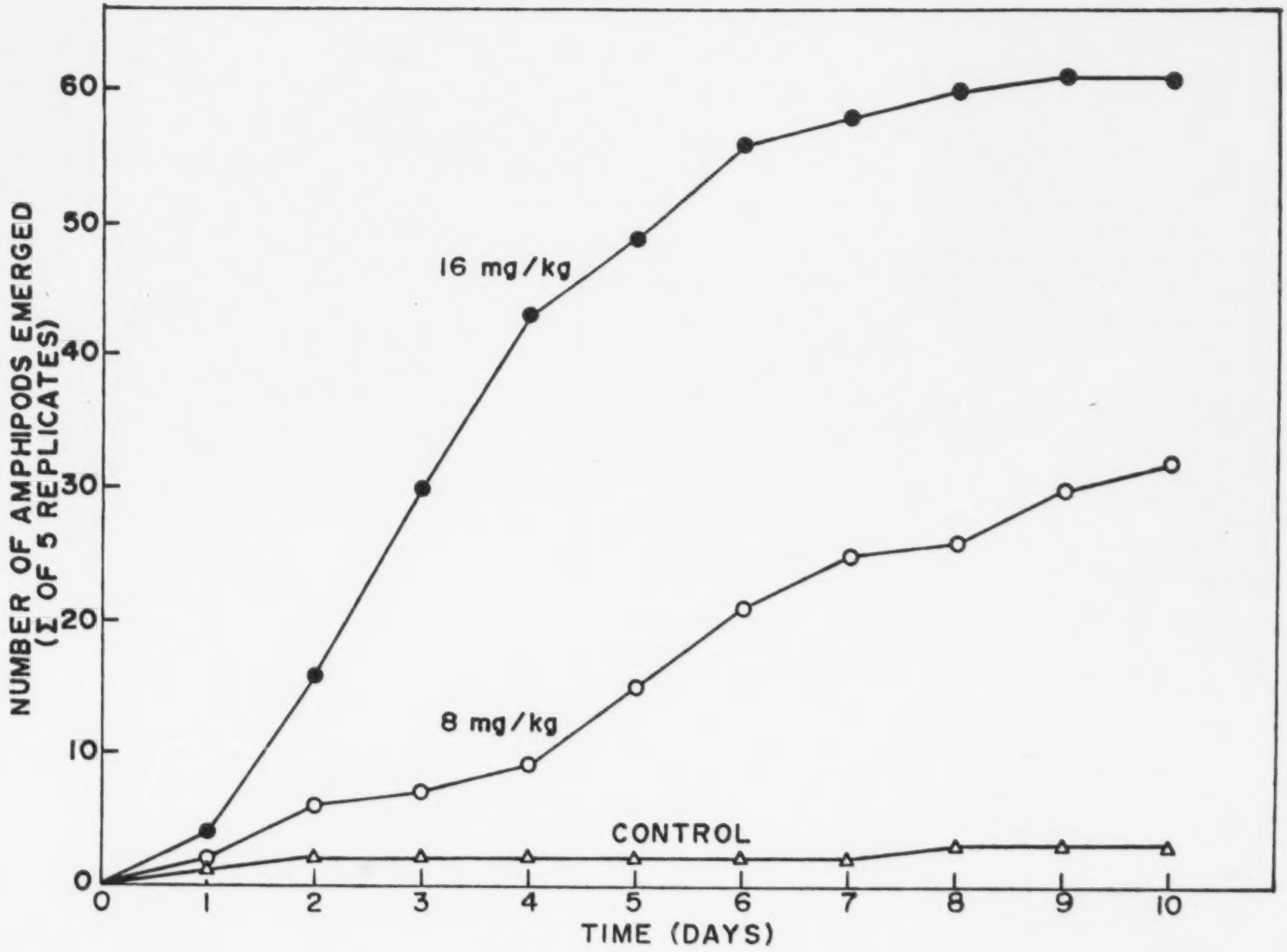
Table 2. Reburial success of Rhepoxynius abronius after ten-day exposure to sediment. Twenty individuals were added to each bioassay sample.

Survival Range	Number of Bioassay Samples	Number of Surviving Amphipods	Failed to Rebury[a]		
			Observed	Expected[b]	Percent
16-20	354	6471	55	164.4	0.85
11-15	134	1768	90	44.9	5.09
6-10	68	555	47	14.1	8.47
1-5	72	181	36	4.6	19.89
Total	628	8975	228	228.0	2.54

[a] $\chi^2 = 398.46^{**}$, $\chi^2_{.01[3]} = 11.3$

[b] Expected numbers based on percent distribution of number of surviving amphipods.

Figure 1. Emergence of Rhepoxynius abronius from control sediment and from sediment containing 8 and 16 mg/kg cadmium (dry weight).


NUMBER OF AMPHIPODS EMERGED
(Σ OF 5 REPLICATES)
60
50
40
30
20
10
0
16 mg/kg
8 mg/kg
CONTROL
0
1
2
3
4
5
6
7
8
9
10
TIME (DAYS)

slower emergence evident in Fig. 1 suggests a response to the gradual debilitating effects of cadmium. Amphipods do not emerge from very high concentrations of cadmium (e.g., 100 mg/kg Cd), presumably because of an essentially instantaneous toxic effect. In nature emergence and migration to uncontaminated sediment may limit debilitating effects of adverse sediment conditions.

Duration of Bioassay

In initial experiments high survival (>90 percent) of R. abronius was obtained for at least 20 days under control conditions. The bioassay duration of ten days was selected to minimize the possibility of starvation. The effect of starvation for ten days on amphipod survival was examined by making sediment azoic by: 1) reducing interstitial water salinity to 0 ppt for eight days [17], and 2) combusting sediment at 550°C for three hours to remove organic matter. Mean survival in five replicates with 20 amphipods for each treatment was not significantly different between control (19.4), freshwater-rinsed (19.0) and combusted (18.6) sediment ($f=0.67$, $p>0.5$). Results of this experiment and the consistently high survival of apparently healthy amphipods for ten days in control and many test sediments indicate that there are no substantial effects of starvation or other laboratory artifacts.

A bioassay duration of less than ten days is not recommended. Many amphipods that emerge from sediment do so after the fourth day of the test (Fig. 1). Experiments on the toxicity of cadmium show that a large proportion of amphipods are alive but unable to rebury after four days. Most of these amphipods are dead after ten days of exposure (Swartz et al., unpublished data).

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Effects of Sediment Characteristics on R. abronius

The effects of sediment characteristics on this species were investigated because little is known about sediment types naturally inhabited and because interpretation of the results of toxicity tests using sediments is difficult, unless controls are appropriate for the tested contaminated sediments. Responses of amphipods must occur as a result of substances within the sediments, such as organic chemicals, metals, sewage, and complex wastes and not because the structure (grain size) differs from that of the control sediment.

Sediment Particle Size - Little is known about the range of sediment types naturally inhabited by Rhepoxynius abronius. We have collected this species from well-sorted, fine sand in Yaquina Bay, Oregon (S/S/C = 97/1/2)[1] and from less well-sorted, sandy silt in the deeper part of Commencement Bay, Washington (S/S/C = 23/69/9).

Swartz et al. [3] included R. abronius in a multispecies bioassay of sediment size fractions ranging from silt to very coarse sand. Although there were no obvious effects of particle size on survival, statistical comparison was not possible because treatments were not replicated. A similar experiment was conducted by adding 20 amphipods to each of five replicates for six treatments; four size fractions of Yaquina Bay, Oregon sediment, an artificial sediment composed of Kaolin clay, and a composite sediment of equal weights of the preceding sediments. Analysis of variance and Student-Newman-Keuls' multiple range test showed that ten day survival in the Kaolin clay was significantly less than the other

[1] S/S/C = Percent Sand/Silt/Clay

treatment means ($F = 38.07$, $P<0.01$). There were no significant differences in survival between the other treatments including the composite sample that contained Kaolin clay.

Bioassays with natural sediment samples collected from various field sites indicate that R. abronius survives well in a broad range of sediment grain sizes (Table 3). The finest sediment that we have tested was collected in Poverty Bay, Washington (S/S/C = 9.7/36.8/53.5). Eighteen of twenty amphipods survived in this sample. Because of the tolerance of R. abronius to a wide variety of sediment types, most toxicity tests may not require that the control sediment exactly match the grain size distribution of the test material. However, a sediment texture control is recommended if the grain size distribution of the test material is outside the ranges given in Table 3.

Organic Enrichment and Redox Potential - R. abronius is tolerant of substantial organic enrichment of sediment. Survival within the control range has been obtained for sediment with a percent total volatile solids (TVS) as high as 18.2 percent (Table 3). A sediment sample with a 39.8 percent TVS was collected beneath a decaying algal mat (Enteromorpha) in a marina at South Beach, Oregon. Enrichment of this sample may have had some effect, but 14 of 20 amphipods survived for ten days.

R. abronius is able to survive ten day exposures to sediment with very low Eh values. Swartz et al. [18] examined the effect on R. abronius of adding sewage sludge to clean sediment. They measured the level of organic enrichment of sludge sediment mixtures and calculated ten day LC50s in relation to the increase in percent total volatile solids (TVS) due to the sludge addition. Eh was measured by inserting an electrode one cm beneath the sediment surface at two day intervals during the ten day bioassay. Mean Eh values of sludge-sediment mixtures for the least toxic (Waldport,

Table 3. Characteristics of natural sediment samples in which bioassay survival was within the normal control range (17-20 survivors).

	Range in Percent Composition	
	Lowest	Highest
Gravel	0.0	36.4
Sand	4.5	97.0
Silt	1.0	78.8
Clay	1.0	53.5
Total Volatile Solids	1.5	18.2

Oregon) and most toxic (Los Angeles County, California) sludge sources tested are given in Table 4. Eh decreased as TVS increased for both sludge sources, but there were major differences in the Eh-survival relation. The Eh value at the TVS LC50 was <+32 mv for Waldport and >+143 mv for Los Angeles County sludge. The tolerance of amphipods to relatively high levels of TVS and low Eh in the Waldport sludge-sediment mixtures indicates that other sediment properties caused mortality in the Los Angeles County mixtures.

Tolerance of R. abronius during acute exposure to sediment with low Eh is also indicated by bioassay data for sediment collected from organically enriched field situations. In May 1980 five sediment samples were collected from a site 0.8 km from the end of the City of Los Angeles (Hyperion) sewage sludge discharge in Santa Monica Bay (33° 56' N, 118° 33' W, S/S/C = 60/33/7, total volatile solids = 14.7 percent). Survival of R. abronius in these samples ($\bar{x}$ = 17.8) was not significantly different from survival in five Yaquina Bay, Oregon control samples ($\bar{x}$ = 19.6, t = 1.72, P < 0.1). However, mean Eh in control bioassay chambers was much greater (+284 mv) than in the Santa Monica Bay bioassay chambers (-91 mv).

Sediment pollution often involves both organic enrichment and contamination by toxic chemicals [20]. Available data indicate that relatively high levels of organic enrichment are not acutely toxic to R. abronius in the absence of chemical contamination. Additional research is needed to discriminate the effects of these two factors on benthic ecosystems.

Temperature and Salinity

The annual temperature range at the R. abronius collection site in Yaquina Bay, Oregon is 7.7-16.1°C. The standard bioassay temperature of 15°C was selected as representative of the upper portion of this range

Table 4. Survival of Rhepoxynius abronius and redox potential of sediment organically enriched with sewage sludge. Twenty amphipods were added to each of five replicates of each treatment.

Percent Increase in Total Volatile Solids from Sludge Addition	Waldport, Oregon		Los Angeles County, California	
	Mean Eh (mv)	Survivors	Mean Eh (mv)	Survivors
0.0	340	17.8	334	18.2
0.125	194	18.2	143	0.4
0.25	136	17.2	119	0
0.50	94	15.2	100	0
1.0	68	14.6	73	0
2.0	32	10	57	0
4.0	13	8	26	0
LC_{50} (Percent Volatile Solids)	2.83		0.07	

because biological effects of sediment contaminants are more likely to occur at higher temperatures. Survival in Yaquina Bay control sediment at 15°C has averaged 19.0 of 20 individuals in 22 experiments. An initial experiment at temperatures above the annual extreme showed mean survival in five replicates of 19.0 amphipods at 17°C and 19.4 amphipods at 19°C. These data indicate that 15°C is not close to the upper thermal tolerance limit of this species.

Restriction of R. abronius to the lower portions of estuaries and offshore habitats suggests that salinity may be a controlling factor in its distribution. To determine the salinity tolerance of the species, five replicates of 20 amphipods were exposed to each of seven salinities ranging from 9.9 to 26.9 ppt. To promote exchange between interstitial and overlying water only a 1-cm deep layer of sediment from the amphipod collection site was added to each bioassay beaker. To avoid an instantaneous salinity stress amphipods were initially added to beakers containing the sediment and 200 ml of 26.9 ppt seawater. Salinity was gradually reduced to experimental conditions by adding dilution water at an appropriate salinity for each treatment over a 4.5 hour period.

R. abronius is very sensitive to low salinity. Mean survival was very high (19.5-19.8 amphipods at experimental salinities of 21.8, 24.3 and 26.9 ppt (Table 5). Reduction in mean survival to 17.4 animals at 18.8 ppt was statistically significant ($p < .05$). No amphipods survived at salinities of 9.9 and 12.3 ppt. To ensure the absence of salinity stress, a standard bioassay salinity of 25 ppt was selected.

Another salinity experiment was conducted to determine if low salinity of interstitial water in test sediment would affect survival even though 25 ppt water was added to bioassay beakers. Salinity of sediment from the amphipod collection site was adjusted by sieving it through buckets

Table 5. Survival of Rhepoxynius abronius at different salinities. Twenty amphipods were added to each of five replicates of each treatment.

Salinity (ppt)	Mean Survival[a]	
9.9	0.0	A
12.3	0.0	A
16.3	3.8	
18.8	17.4	
21.8	19.8	B
24.3	19.5	B
26.9	19.8	B

[a] Differences between means were highly significant ($F=138.37$, $p < 0.01$). Any two means not connected by the same vertical line are significantly different ($p < 0.05$) as determined by Student-Newman-Keuls' multiple range test.

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containing water at six salinities ranging in 5 ppt intervals from 0 to 25 ppt. The normal 2-cm depth of test sediment was added to five replicates for each salinity treatment (Appendix A).

Mean survival at interstitial salinity < 15 ppt was significantly less than that at 25 ppt (Table 6). The difference between survival at 20 ppt and 25 ppt was barely non-significant ($p = 0.054$). Clearly the higher salinity overlying water did not effectively penetrate test sediment in the bioassay beakers. Conservatively, interstitial salinity of test sediment should be at least 25 ppt before salinity effects on survival can be discounted.

The sensitivity of R. abronius to salinity effectively limits the application of this test procedure to field sediment samples collected from the coastal zone and higher salinity portions of estuaries. Attempts to raise interstitial salinity by adding or sieving into higher salinity water are likely to change the toxicological properties of the sample.

Field Validation

The purpose of a field validation of a bioassay procedure is to determine if results obtained under laboratory conditions are relevant to pollution effects on real ecosystems. The ecological relevance of the R. abronius bioassay has been tested along two well-defined pollution gradients located (1) on the Palos Verdes shelf near the White's Point, California sewage outfall and (2) Commencement Bay, Washington and the industrialized waterways adjacent to the Bay [9, 19, 20]. The condition of macrobenthic communities and the toxicity of sediment were examined along both gradients. In each case amphipods, including phoxocephalids, were diverse and abundant in areas of relatively low contamination. Sediment from these sites was not toxic to R. abronius as determined by the bioassay method described in this report. The condition

Table 6. Survival of Rhepoxynius abronius at different interstitial water salinities. Salinity of water above the sediment surface was 25 ppt in all treatments. Twenty amphipods were added to each of five replicates of each treatment.

Interstitial Water Salinity	Mean Survival[a]
0.0	11.0
5.0	11.2
10.0	13.8
15.0	14.4
20.0	15.4
25.0	19.0

[a] Differences between means were highly significant ($F=5.49$, $p < 0.01$). Any two means not connected by the same vertical line are significantly different ($p < 0.05$) as determined by Student-Newman-Keuls' multiple range test.

of the amphipod assemblage decreased at sites progressively closer to the sources of pollution. At stations within 3 km of the White's Point sewage outfall amphipods were absent and survival of R. abronius in the bioassay was significantly less than in control sediment. In the Commencement Bay waterways amphipods were rare and sediment samples were often highly toxic to R. abronius.

Results of these two investigations show that along gradients of increasing sediment contamination the toxicity of sediment to R. abronius increases as phoxocephalids and other amphipods disappear from the benthos. This may not constitute an unequivocal field validation, because other sediment characteristics (grain size, organic enrichment, etc.) also change along these gradients, however, these results do indicate that amphipod bioassay results correspond with the effects of pollution on natural communities.

DISCUSSION

Modification of the Method for Other Amphipod Species

The procedure described in Appendix A for determining the acute toxicity of sediment to R. abronius may be applicable to many other infaunal amphipods. The method is very similar to the sediment bioassay developed by Gannon and Beeton [21] for the freshwater amphipod, Pontoporeia affinis. Sediment bioassays with phoxocephalids or other marine amphipods have been conducted successfully by several investigators using methods similar to those of Appendix A [22-24].

The tolerance of a prospective test species to natural variations in sediment characteristics must be established before responses can be ascribed to contaminant effects. Key parameters include particle size distribution, organic enrichment, and interstitial water salinity. Choice

of the scale of the bioassay chamber, density of test organisms, temperature, salinity, and control sediment should be based on conditions at the natural habitat of the species.

Inter-Method Comparisons

Independent investigations of sediment toxicity indicate a correspondence between results obtained using the Appendix A procedure and other sediment bioassay methods. As part of EPA's Chesapeake Bay Program [25] we conducted bioassays of sediment from the Elizabeth River, Virginia. Toxicity was greatest in five samples from the lower portion of the Southern Branch of the Elizabeth River. Alden and Young [26] conducted bioassays of the toxicity of sediment from the Elizabeth River to the grass shrimp Palaemonetes pugio. The toxicity they reported was mainly associated with the suspended solids phase of sediment from the entrance and lower portion of the Southern Branch. In both investigations relatively low toxicity was caused by sediment from the entrance to the Elizabeth River or the upper portion of the Southern Branch.

Chapman et al. [22] used several sediment bioassays to document the occurrence of sediment toxicity in Puget Sound. These included acute mortality tests with an oligochaete (Monopylephorus cuticulatus), amphipod (Eogammarus confervicolus), and fish (Gasterosteus aculeatus); tests for respiration anomalies in M. cuticulatus; and genotoxicity tests for chromosomal damage to gonadal cells of rainbow trout (Salmo gairdneri). The sublethal tests (respiration and genotoxicity) were more sensitive than the acute mortality tests. Chapman et al. [22] reported a "good correspondence" between their determination of the spatial distribution of sediment toxicity in the Hylebos and Blair Waterways and that determined from R. abronius bioassays by Swartz et al. [9]. Chapman et al. [22] also found a good correspondence between their bioassay results for sediment

from the Duwamish River and Elliott Bay dumpsite and earlier bioassays by Shuba *et al.* [27] with *Palaemonetes* and Swartz *et al.* [3] with *R. abronius* and *Macoma inquinata*.

Many sediment bioassay methods have been generated since the EPA/COE Implementation Manual [4] for evaluating toxicity of dredged materials was published in 1977 [28-38]. These involve a great phylogenetic variety of test species. Response criteria range from subcellular anomalies to effects on benthic community structure. There is an important research need for comparisons of the sensitivity and ecological relevance of the different methods. However, no single technique can meet all sediment bioassay requirements. Complementary methods are needed to address problems of acute toxicity, bioaccumulation, and sublethal effects on various levels of biological organization.

Applications in Regulatory and Research Programs

There are several potential applications of the Appendix A procedure and other sediment bioassays in regulatory and research programs. The acceptability of dredged material proposed for ocean disposal can be evaluated, in part, by sediment bioassay. The Corps of Engineers is currently implementing such bioassays in its dredged material permit program. Ocean disposal permit programs for complex wastes like sewage sludge and drilling muds could also utilize sediment bioassays. The feasibility of determining the relative toxicity of solid wastes has been established [18, 39].

Sediment quality criteria analogous to water quality criteria could be used to estimate safe concentrations of toxic materials in sediment. The difficulty of this concept is that the toxicity and bioavailability of contaminants are highly dependent on the geochemical properties of the sediment.

The spatial or temporal distribution of sediment toxicity can be rapidly and easily determined by sediment bioassays. These distributions can be used to identify areas of relatively high toxicity and to monitor toxicity patterns at marine disposal sites. Benthic communities at sites where sediment is acutely toxic to R. abronius are often greatly affected by sediment contamination [20]. The absence of acute sediment toxicity does not demonstrate the absence of benthic degradation. Sediment bioassays should, therefore, not be the sole criteria of the "health" of benthic ecosystems [20].

Sediment bioassays have many applications as research tools. Interactions between contaminants can be assessed in relation to toxicity of materials added to sediment. The toxicity of sediment samples collected in the field can be compared to chemical and geological sediment characteristics and the condition of benthic communities to give a more comprehensive analysis of sediment quality.

CONCLUSIONS

The acute toxicity of marine sediment can be accurately determined and sediments ranked according to relative toxicity through acute, static bioassays with the phoxocephalid amphipod, Rhepoxynius abronius. The major limitation is that R. abronius is sensitive to interstitial water salinity less than 25 ppt. Unless interstitial salinity can be adjusted, bioassays

of sediment collected in the field are limited to samples from the lower portion of estuaries and the coastal zone. Additional research is needed to discriminate effects of organic enrichment and toxic chemicals on R. abronius.

The advantages of the bioassay include its simplicity, short duration, and precision for statistical analyses. The bioassay species is an ecologically important member of the benthic community. It is tolerant of a wide range of natural sediment characteristics, but is sensitive to sediment contaminants. The efficacy of the R. abronius bioassay has been demonstrated in both experimental addition of toxic materials to clean sediment and field assessments of the spatial distribution of sediment toxicity. Field validation of the method has shown a correspondence between sediment toxicity and the occurrence of amphipods along pollution gradients.

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REFERENCES

[1] Goldberg, E. D. in Assimilative Capacity of U. S. Coastal Waters for Pollutants, E. D. Goldberg, Ed., "Federal Plan for Ocean Pollution Research Development and Monitoring, FY 1981-1985," NOAA Working Paper No. 1, National Oceanic and Atmospheric Administration, Boulder, Colorado, 1979.

[2] Chapman, P. M. and Long, E. R., Marine Pollution Bulletin, Vol. 14, No. 3, March 1983, pp. 81-84.

[3] Swartz, R. C., DeBen, W.A. and Cole, F. A., Journal Water Pollution Control Federation, Vol. 51, No. 5, May 1979, pp. 943-950.

[4] Environmental Protection Agency/Corps of Engineers, "Ecological Evaluation of Proposed Discharge of Dredge Material into Ocean Water," Environmental Effects Laboratory, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1977.

[5] Oliver, J. S., Slattery, P. N., Hulberg, L. W. and Nybakken, J. W., "Patterns of Succession in Benthic Infaunal Communities Following Dredging and Dredged Material Disposal in Monterey Bay," Technical Report D-77-27, U. S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1977.

[6] Barnard, J. L., Proceedings of the Biological Society of Washington, Vol. 92, No. 2, Aug. 1979, pp. 368-379.

[7] Barnard, J. L. and Barnard, C. M., "The genus Rhepoxynius (Crustacea: Amphipoda: Phoxocephalidae) in American Seas," Smithsonian Contributions to Zoology, No. 357, Smithsonian Institution Press, Washington, D.C., 1982.

[8] Barnard, J. L., Allan Hancock Pacific Expeditions, Vol. 18, No. 3, May 1960, pp. 175-368.

[9] Swartz, R. C., DeBen, W. A., Sercu, K. A. and Lamberson, J. O. Marine Pollution Bulletin, Vol. 13, No, 10, Oct. 1982, pp. 359-364.

[10] Hannan, C. A., Hulberg, L. W., Mawn, K. M., Kellog, M. G. and Nybakken, J. W., "Life History Analyses of Marine Benthic Invertebrates," Publication No. 63, California State Water Resources Control Board, Sacramento, California, 1979.

[11] Boesch, D. F., in Ecological Stress and the New York Bight: Science and Management, G. F. Mayer, Ed., Estuarine Research Federation, Columbia, South Carolina, 1982, pp. 543-568.

[12] Manzanilla, E. and Cross, J. N., 1981-1982 Biennial Report, Southern California Coastal Water Research Project, Long Beach, California, 1982, pp. 119-124.

[13] Oliver, J. S., Oakden, J. M. and Slattery, P. N., Marine Ecology Progress Series, Vol. 7, No. 2, Feb. 1982, pp. 179-184.

[14] Mearns, A. J. and Word, J. Q., in Ecological Stress in the New York Bight: Science and Management, G. F. Mayer, Ed., Estuarine Research Federation, Columbia, South Carolina, 1982, pp. 495-512.

[15] National Marine Fisheries Service, "The Effects of Waste Disposal in the New York Bight. Final Report. Section 2: Benthic Studies," NTIS Report No. AD739532, Sandy Hook Laboratory, Highlands, New Jersey, 1972.

[16] Snedecor, G. W., Statistical Methods, 5th ed., The Iowa State University Press, Ames, Iowa, 1956, pp. 60-62.

[17] Wilson, W. H., Jr., Ecology, Vol. 64, No. 2, April 1983, pp. 295-306.

[18] Swartz, R. C., Schults, D. W., Ditsworth, G. R. and DeBen, W. A., Toxicity of Sewage Sludge to Rhepoxynius abronius, a Marine Benthic Amphipod, in preparation.

[19] Swartz, R. C., Schults, D. W., Ditsworth, G. R., DeBen, W. A. and Cole, F. A., Estuaries, Vol. 4, No. 3, Sept. 1981, p. 258.

[20] Swartz, R. C., Schults, D. W., Ditsworth, G. R., DeBen, W. A. and Cole, F. A., "Sediment Toxicity and the Distribution of Marine Macrobenthos Near a Large Sewage Outfall," manuscript in preparation.

[21] Gannon, J. E. and Beeton, A. M. Journal of the Water Pollution Control Federation, Vol. 43, No. 3, Mar. 1971, pp. 392-398.

[22] Chapman, P. M., Vigers, G. A., Farrell, M. A., Dexter, R. N., Quinlan, E. A., Kocan, R. M. and Landolt, M., "Survey of Biological Effects of Toxicants upon Puget Sound Biota I. Broad-scale Toxicity Survey," NOAA Technical Memorandum OMPA-25, National Oceanic and Atmospheric Administration, Boulder, Colorado, 1982.

[23] Pierson, K. B., Nichols, J. W., McDowell, G. C. and Nakatani, R. E., "Grays Harbor, Washington, Dredged Sediments: An Assessment of Potential Chemical Toxicity and Bioaccumulation," Fisheries Research Institute, University of Washington, U. S. Army Corps of Engineers, Seattle, 1983.

[24] Personal Communication; Robert Reid, Frederika Ott, Joseph Cummins, John Scott.

[25] Chesapeake Bay Program Technical Studies: A Synthesis, U. S. Environmental Protection Agency, Washington, D. C., 1982.

[26] Alden, R. W., III and Young, R. J., Jr., Archives of Environmental Contamination and Toxicology, Vol. 11, No. 5, Sept. 1982, pp. 567-576.

[27] Shuba, P. J., Tatem, H. E. and Carroll, J. H., "Biological Assessment Methods to Predict the Impact of Open-water Disposal of Dredged Material," Dredged Material Research Program, Technical Report D-78-50, Department of the Army, Vicksburg, Mississippi, 1981.

[28] Shuba, P. J., Petrocelli, S. R. and Bentley, R. E., "Considerations in Selecting Bioassay Organisms for Determining the Potential Environmental Impact of Dredge Material," Technical Report EL-81-8, U. S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1981.

[29] Hansen, D. J. and Tagatz, M. E., in Aquatic Toxicology, ASTM STP 707, J. G. Easton, P. R. Parrish and A. C. Hendricks, Eds., American Society for Testing and Materials, 1980, pp. 40-57.

[30] Hardy, J. T., Schmidt, R. L. and Apts, C. W., Bulletin of Environmental Contamination and Toxicology, Vol. 27, No. 6, Dec. 1981, pp. 798-805.

[31] Peddicord, R. K., in Contaminants and Sediments, Vol. I, Fate and Transport, Case Studies, Modeling, Toxicity, R. A. Baker, Ed., Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1980, Chapter 25, pp. 501-536.

[32] Tatum, H. E., in Contaminants and Sediments, Vol. I., Fate and Transport, Case Studies, Modeling, Toxicity, R. A. Baker, Ed., Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1980, Chapter 26, pp. 537-549.

[33] Rubinstein, N. I., in Marine Pollution: Functional Responses, W. B. Vernberg, F. P. Thurberg, A. Calabrese and F. J. Vernberg, Eds., Academic Press, Inc., New York, 1979, pp. 341-351.

[34] Pearson, W. H., Woodruff, D. L., Sugarman, P. C. and Olla, B. L., Estuarine, Coastal and Shelf Science, Vol. 13, No. 4, Oct. 1981, pp. 445-454.

[35] Tietjen, J. H. and Lee, J. J., "The Use of Free-living Nematodes as a Bioassay for Estuarine Sediments," Environmental Pollution (Series A), in press.

[36] Lee, G. F., Piwoni, M. D., Lopez, J. M., Mariani, G. M., Richardson, J. S., Homer, D. H. and Sale, F., "Research Study for the Development of Dredged Material Disposal Criteria," Contract Report D-75-4, U. S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1975.

[37] Jones, R. A. and Lee, G. F., "Evaluation of the Elutriate Test as a Method of Predicting Contaminant Release During Open-water Disposal of Dredged Sediments and Environmental Impact of Open-water Dredged Material Disposal, Vol. 1: Discussion," Technical Report D-78-45, U. S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1978.

[38] Møhlenberg, F. and Kiørboe, T., Marine Pollution Bulletin, Vol. 14, No. 2, Feb. 1983, pp. 57-60.

[39] Tagatz, M. E. and Tobia, M., Estuarine and Coastal Marine Science, Vol. 7, No. 4, Oct. 1978, pp. 401-407.

Appendix A: Procedure for Amphipod Sediment Toxicity Bioassay

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I. INTRODUCTION

This appendix provides detailed guidance for performing sediment toxicity bioassays with marine infaunal amphipods. The method is a static, ten-day test that addresses the toxicity relative to controls of sediment samples collected from the field or prepared in the laboratory. Instructions are given for the collection of the test species, sediment and seawater; followed by the procedure for initiating, monitoring and terminating the bioassay. Aspects of experimental design that are specific to the amphipod test are discussed fully and references given to more general statistical methods for analyzing results.

II. BIOASSAY SPECIES

A. Species Selection - Rhepoxynius abronius (Phoxocephalidae: Amphipoda) is the preferred species, although other phoxocephalids or free-burrowing amphipods may be acceptable. Environmental requirements of the test species determine certain experimental conditions. Standard bioassay temperature and salinity for R. abronius are 15°C and 25 ppt . If another species is used, its temperature and salinity tolerance limits must be established to determine standard bioassay conditions.

B. Field Collection - Amphipods are collected three to four days before the bioassay is initiated. They are obtained by benthic grabs (e.g., van Veen, Smith-McIntyre) or small dredges. If a dredge is used, a short haul (10 m) will minimize potential damage to the animals during collection. About one third more animals than are required for the bioassay are collected.

Surface and bottom seawater salinity and temperature are measured at the collection site. Sediment temperature is recorded from the first and last dredge sample. Samples for geochemical analyses are taken as a

composite of all dredge samples. Contents of the dredge are then gently washed into a container using seawater of similar temperature and salinity to that at the collection depth. Samples that show evidence of contamination (e.g., oil sheen) are rejected. Amphipods will typically bury in the sediment and if necessary can be held in the containers for several hours (at the bottom temperature) prior to sieving. It is preferable to minimize the delay from collection to sieving. To avoid handling stress, each dredge sample is placed in a separate container.

C. Sieving - A 0.5 mm sieve is used to remove R. abronius from sediment. Gentle sieving is essential to reduce handling stress. The sieve is placed in a large tub filled with seawater at ambient collection site bottom salinity and temperature. The entire contents of each holding container, including water, are washed through the sieve using seawater pumped at low pressure through a fan spray nozzle. The sieve can be shaken gently, but the bottom of the screen must be beneath the water surface at all times. Material retained on the screen is washed into buckets for transport to the laboratory for sorting. Large pieces of detritus and obvious predators are discarded. If there is a delay of more than one hour before sorting begins, the buckets should contain enough sieved sediment to allow the amphipods to bury. The buckets must be kept at collection site temperature. Aeration may be necessary.

D. Sorting - An aliquot of detritus or sediment containing amphipods is placed in a sorting tray. Healthy, active animals are removed with a bulb pipette (5-mm opening) and placed in 10 cm diameter finger bowls filled with 25 ppt seawater and a 2 cm deep layer of 0.5 mm-sieved collection site sediment. Twenty amphipods are transferred to each bowl and enough bowls are prepared to provide at least one third more specimens than are required for the bioassay. For R. abronius, seawater temperature

during sorting must not exceed 18°C. Filled finger bowls are submerged in holding tanks supplied with flowing water or aeration where temperature and salinity approximate bioassay conditions. If temperature and salinity adjustments are necessary they should be made gradually. Healthy R. abronius will remain in the finger bowl sediment and can be retrieved easily when the bioassay is set up.

III. BIOASSAY SEDIMENT

This section addresses the collection and preparation of control and test sediment. Control sediment is collected simultaneously with the amphipods and therefore provides a non-toxic reference sediment. The test sediment is either collected from potentially contaminated field sites or is prepared by addition of toxic materials to or other manipulations of the control sediment. The objective of the bioassay is to statistically compare mortality and sublethal effects in control and test sediments.

A. Control Sediment - R. abronius typically inhabits well-sorted, fine sand. Suggestions for sieving and settling may have to be adjusted for other sediment types.

A separate container is set up during sieving to collect control sediment. Approximately 0.25 l of control sediment is collected for each bioassay beaker. This sediment is sieved twice, first, to remove the test species and other macrobenthos, and second, to adjust interstitial water salinity. The entire contents of one or more dredged samples, including water and suspended particulate matter, are sieved through a 0.5-mm screen without allowing overflow from the container. After the first sieving, sediment is allowed to settle for at least four hours. Overlying water is then decanted and the sediment resieved through a 0.5-mm screen into water of the bioassay salinity (25 ppt for R. abronius). Again, the

sediment is allowed to settle for at least four hours, preferably overnight, overlying water is decanted and the control sediment is held at 4°C until the bioassay chambers are prepared.

B. Test Sediment - If the test sediment is collected from potentially contaminated field sites, a benthic grab rather than dredge is used to minimize disruption of the sample. Cores are used to collect a sample from the upper 2 cm, or desired layer, of the test sediment. This operation is facilitated if plates on the back of the grab can be opened to expose an undisturbed sediment surface. Approximately 0.25 l of test sediment are necessary for each bioassay beaker. Test sediment should be stored at 4°C for not more than five days before the bioassay begins. Longer storage or freezing may change sediment properties, and should be avoided, if possible.

Additional samples may be taken from the grab for other kinds of sediment analyses. These may include particle size distribution, organic enrichment (total volatile solids, biological oxygen demand, or total organic carbon), metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, interstitial water salinity, and sediment temperature. Qualitative description of the sediment may include color, odor, texture, presence of plants, animals, tracks and burrows. The remainder of the sediment can be sieved to provide a macrobenthos sample.

The natural geochemical properties of test sediment collected from the field must be within the tolerance limits of the test species. R. abronius may be adversely affected by salinity stress if the interstitial water salinity is below 25 ppt. This species is tolerant of a broad range of sediment types. However, controls for the effects of particle size distribution are recommended if the clay content exceeds 50 percent or the gravel content exceeds 35 percent.

Test sediment can also be prepared in the laboratory by manipulating the properties of control sediment. This can include adding toxic chemicals or complex waste mixtures (e.g., sewage sludge). Natural sediment characteristics such as interstitial salinity, temperature, particle size, and organic content can be modified to determine the requirements of the test species. Techniques for these manipulations are discussed by Swartz et al. [1] and Ditsworth and Jones [2].

IV. BIOASSAY SEAWATER

Seawater used in the bioassay is maintained at a salinity and temperature characteristic of the amphipod collection site. For R. abronius collected from Yaquina Bay, appropriate seawater conditions are 15°C and 25 ppt. Other conditions may be acceptable, but at the present state of knowledge, we recommend that salinity should be $\geq$ 25 ppt and temperataure $\leq$ 19°C. If a series of experiments is planned, test temperature and salinity should be the same throughout the series. The bioassay seawater must be uncontaminated which may necessitate collection of seawater at the amphipod collection site. The quantity of seawater required is dependent on sieving and holding needs and the number of bioassay chambers.

Bioassay seawater is passed through a 0.45 µm pore diameter filter. If necessary, salinity is reduced by addition of deionized distilled water or raised by addition of clean oceanic water or reagent grade chemicals [3]. Seawater is prepared within two days of the bioassay and stored in covered, clean containers at the bioassay temperature.

For certain applications the experimental design may require use of seawater from the test sediment collection site. In other instances, experimental treatments may involve manipulation of bioassay seawater characteristics.

V. FACILITIES AND EQUIPMENT

The bioassay chamber is a standard 1 litre glass beaker (10 cm internal diameter) covered with an 11.4 cm diameter glass watchglass. The beakers are placed in a shallow water bath with overhead aeration source. Aeration to each beaker is provided through a 1-ml glass pipette that extends between the beaker spout and watchglass to a depth not closer than 2 cm from the sediment surface. Air is bubbled into the beakers at a rate that does not disturb the sediment. The bioassay temperature is maintained by either the water bath or room temperature control.

All glassware is cleaned by washing with laboratory detergent, rinsing in turn with distilled water, ten percent HNO_3, and distilled water, and baking for 8 hours at 300°C. Large plastic containers and plastic sieves used for preparation and storage of sediment and seawater are preconditioned initially by soaking for 24 hours in seawater and rinsed after each use with clean seawater. They are used only for bioassays and stored in a clean room. Sieves and containers used to collect and store amphipods, seawater and control sediment are kept separate from those used for test sediment.

VI. BIOASSAY PROCEDURE

A. Initiation - The day before the bioassay is initiated, each test sediment sample is mixed within its storage container and an aliquot sufficient to make a 2 cm deep layer is added to a bioassay beaker. In the

case of replicate bioassay samples, the weight of sediment necessary to make a 2 cm deep layer (175 ml) in the first beaker is added to the other replicates. The same procedure applies to control sediment. Treatments are randomly assigned to prenumbered bioassay beakers.

The sediment aliquot in the beaker is settled by smoothing with a teflon spoon and bubbles are removed by tapping the beaker against the palm of the hand. A disk cut from black plastic sheeting (attached to a nylon string for removal) is placed on the sediment surface. This minimizes sediment disruption as 750 ml of bioassay seawater are added. The disk is removed and rinsed in bioassay water between beakers and changed between treatments. The beakers are covered with watchglasses, put into the 15°C water bath, and aerated. Tap water is added to the water bath up to the 700 ml mark on the beakers. The beakers are allowed to equilibrate overnight to bioassay conditions. Normal room lighting is left on continuously during the bioassay. If the experimental design requires monitoring of sediment chemistry (e.g., metals, total volatile solids, Eh, etc.), additional beakers must be set up for this purpose. Monitoring the quality of seawater overlying the sediment can be accomplished in the bioassay beakers without disturbing the sediment. Temperature is recorded from a thermometer maintained in a separate beaker containing control sediment and bioassay water, but no amphipods.

On the day the bioassay is initiated, amphipods are distributed among all beakers so that each receives 20 individuals. It is usually not logistically possible to distribute amphipods to all beakers at the same time, so it is necessary to select a portion of the beakers (as many as 15) to be processed together. The exact number of beakers to receive amphipods at one time is dependent on the size and design of the experiment. If

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treatments are replicated, at least one replicate from each treatment, including control sediment, is processed at a time. If treatments are not replicated, selection is random.

Amphipods are removed from the holding sediment by use of a 1.0-mm sieve and transferred to sorting trays. About one third more fingerbowls are removed from the holding tank at one time than are required for the number of beakers. This allows selection of active, healthy animals for the bioassay. Amphipods are removed from the sorting tray and sequentially distributed among clean 10 cm fingerbowls each containing 150 ml of bioassay seawater without sediment. The number of amphipods distributed to each fingerbowl is recounted by transferring them to a separate fingerbowl.

Animals are added to the bioassay beakers by placing a black plastic disk on the seawater surface and gently pouring the entire contents of the fingerbowl into the beaker. The fingerbowl is washed with bioassay water to remove adhering amphipods. The seawater level is brought up to 950 ml with bioassay water, and the disk is removed and rinsed between samples. Amphipods are allowed to bury in the sediment and any that are floating on the seawater surface are pushed down with the edge of the beaker cover. After one hour amphipods that have not buried are removed and replaced. Normally, less than one percent of the animals will fail to bury in one hour.

B. Monitoring - If samples for chemical analysis are desired, seawater and sediment samples can be taken from beakers at the initiation of the bioassay. A small quantity of seawater can be removed from the bioassay beakers, but chemistry beakers have to be sacrificed to obtain sediment samples. This is accomplished by siphoning the overlying seawater without disturbing the sediment surface and then taking appropriate sediment aliquots for chemical analyses. It is not necessary to add amphipods to chemistry beakers that are sacrificed at the initiation of the

bioassay, but amphipods are added to those sacrificed later. Certain sediment and water quality parameters (e.g., DO, pH, Eh) can be monitored by inserting analytical probes into the chemistry beakers.

During the course of the bioassay certain observations are made daily. Temperature in the beaker set up for this purpose is monitored. Lighting and aeration systems are checked. Each beaker is carefully examined, but not disturbed except for the temporary removal of the aeration pipette and watchglass. Notes are made on sediment appearance and any unusual conditions. The number of amphipods that have emerged from the sediment, either floating on the water surface or lying on top of the sediment, is counted. Amphipods that have emerged are not removed, even if they are dead. These data are used to document the temporal pattern of emergence.

C. Termination - The bioassay is terminated after ten days of exposure. After daily observations are recorded the bioassay beakers are sacrificed by sieving the contents through a 0.5-mm screen. Material retained on the screen is placed in clean bioassay water in a sorting tray. The numbers of live and dead amphipods are recorded. The sum of these numbers may not always equal 20 because of decomposition. An amphipod is counted as alive if there is any sign of life, e.g., pleopod twitch observed under magnification. Live amphipods are transferred to 10-cm diameter fingerbowls containing a 2-cm deep layer of control sediment and filled with clean bioassay water. The number of individuals that are able to rebury in one hour is recorded. These data are used to document sublethal effects of toxicants on behavior.

VII. EXPERIMENTAL DESIGN

A. Logistics - A typical sediment bioassay involves about 50-60 bioassay beakers. Collection and preparation of animals, sediment, and seawater requires at least four people for two days. Three or four people are required on the days experiments are initiated and terminated. One person can monitor the experiment in progress.

B. Controls - Five replicates of the amphipod collection site control sediment are included in all bioassays regardless of whether test sediments are replicated. This allows comparisons among experiments and among laboratories of the validity of the procedures used in individual investigations. At least 90 percent of the amphipods in the control replicates should survive. The design of field surveys may include an additional control involving five replicate samples from an area believed to be free from sediment contamination. This provides a site-specific basis for comparison of potentially toxic and nontoxic conditions. Experiments in which contaminants are added to sediment may require control replicates to determine effects of solvent addition.

C. Response Criteria - Three response criteria are examined in all bioassays; emergence from sediment, survival and ability to rebury. Survival after ten days of exposure is the primary criterion of toxicity. Data on the ability of the amphipods to rebury in clean sediment are used to detect biologically significant sublethal effects. Emergence data are used to monitor a behavioral response of the amphipods during the ten day exposure. Each of the three response criteria must be monitored in a "blind" fashion, i.e., the observer must have no knowledge of the treatment of the sediment in the beakers. This is accomplished through randomization of beaker numbers.

D. Field Survey Design - Field surveys can be designed to provide either a qualitative reconnaissance of the distribution of sediment toxicity or a quantitative statistical comparison of toxicity among stations. The objective of a reconnaissance survey is to identify sites of potential toxic conditions that warrant further study. It is often conducted in areas where little is known of contamination patterns. Samples are usually not replicated from stations to allow for maximum spatial coverage. The lack of replication precludes statistical comparisons, but samples of concern can be identified if mortality exceeds the control range. For R. abronius, survival of less than 17 of 20 individuals would indicate possible toxicity.

The objective of a quantitative sediment toxicity survey is to test for statistically significant differences in survival among control and test sediments. Five replicates are taken at each station in the survey. Depending on the design of the survey, the five replicates can represent separate samples from different grabs taken at the same station, separate samples from the same grab, or split samples of composited sediment from one or more grabs. Station locations may be distributed along a known pollution gradient, in relation to the boundary of a disposal site, or at sites identified as being potentially toxic in a reconnaissance survey. Comparisons can be made in both space and time.

Sediment toxicity surveys are usually part of more comprehensive analyses of biological, chemical, geological and hydrographic conditions. A useful summary of field sampling design is presented by Green [4]. Many statistics texts describe methods for comparing differences in mean toxicity between stations and for correlating toxicity with other

ecological parameters (e.g., analysis of variance, multiple range tests) [5-6]. The EPA/COE [7] Implementation Manual gives examples of statistical analysis of sediment bioassay data.

E. Laboratory Experiments - Laboratory experimental bioassays can address a great variety of sediment and water quality manipulations. This can include natural limiting factors such as salinity, temperature, particle size distribution, dissolved oxygen, sediment organic content, etc. Toxic concentrations of specific chemical and complex waste mixtures can be determined by adding such materials to control sediment. Interactions between stresses can also be examined. The most basic experimental design is to establish a dose-response relation and estimate the concentration or level of stress necessary to affect 50 percent of the test organisms. The LC50 is the concentration that causes mortality of half of the animals in ten days. The concentration that is effective in causing half of the amphipods to be unable to rebury after ten days of exposure is referred to as an EC50. The EC50 concept is not applicable to emergence data because amphipods may die in sediment without emerging. The dose-response relation can also be used to estimate no-effect or safe levels of limiting factors and toxic materials.

Experimental sediment bioassays to establish an LC50 usually involve four or five experimental concentration levels and the control. All treatments have five replicates. In range-finding bioassays a logarithmic concentration series is used to give a rough estimate of the LC50. In definitive bioassays the concentration intervals between treatments are greatly reduced. At least one treatment should give a partial response below the LC50, and one above the LC50. Statistical procedures for the LC50 estimate are given in APHA [8], Finney [9], and Rand [10].

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VIII. CONCLUSIONS

The amphipod bioassay described in this appendix has been used successfully to determine the relative toxicity of a great variety of contaminated sediment samples. Required facilities and equipment are inexpensive and available in most biological laboratories. Perhaps the only difficulty of the method lies in its logistical complexity. The procedures described here should be followed very carefully, especially those that are intended to minimize collection and handling stress on the amphipods.

IX. REFERENCES

[1] Swartz, R. C., Schults, D. W., Ditsworth, G. R. and DeBen, W. A., "Toxicity of Sewage Sludge to Rhepoxynius abronius, a Marine Benthic Amphipod," in preparation.

[2] Ditsworth, G. R. and J. K. P. Jones, "Synthesizing Laboratory Benthic Bioassay Substrates: A Method," in preparation.

[3] "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians," in Annual Book of ASTM Standards, E729-80, American Society for Testing and Materials, Philadelphia, Pennsylvania, 1980, p. 21.

[4] Green, R. H., Sampling Design and Statistical Methods for Environmental Biologists, Wiley, New York, 1979, pp. 1-257.

[5] Snedecor, G. W., Statistical Methods, 5th ed., The Iowa State University Press, Ames, Iowa, 1956, pp. 60-62.

[6] Sokal, R. R. and F. J. Rohlf, Biometry, 2nd ed., W. H. Freeman and Company, San Francisco, California, 1981, pp. 1-776.

[7] Environmental Protection Agency/Corps of Engineers, "Ecological Evaluation of Proposed Discharge of Dredge Material into Ocean Water," Environmental Effects Laboratory, U. S. Army Engineer Waterways Experiment Station, Vicksburg, Mississipi, 1977.

[8] American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 15th ed., APHA, Washington, D.C., 1980, pp. 1-1134.

[9] Finney, D. J., Probit Analysis, 3rd ed., Cambridge University Press, Cambridge, England, 1971, pp. 1-333.

[10] Rand, G. M., in Introduction to Environmental Toxicology, F. E. Guthrie and J. J. Perry, Eds., Elsevier North Holland, Inc., New York, 1980, Chapter 30, pp. 390-403.